REMARKS

Status of the Claims

Claims 1-4, 8 and 13-56 are currently pending. Claims 3-4 and 34-55 are withdrawn as being drawn to non-elected inventions. Claims 1-2, 8, 13-33 and 56 were examined and rejected.

In this response, claims 1, 13, 15, 27-29, 31 and 33 have been amended to clarify the invention and to correct certain typographical errors. No new matter has been added. Entry of the amendments and reconsideration of the claims in view of the following comments are respectfully requested.

Rejection under 35 U.S.C. § 112, ¶ 2

Claims 1-2, 8, 13-33 and 56 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Office states that the specification fails to define the phrase "undesirable constituents" in claim 1, making the meaning of the phrase unclear.

Claims 1, 13 and 31 have been amended to replace the term "undesirable" with "unbound" to clarify that these claims merely refer to magnetic separation of magnetic bead-bound cells, organelles or viruses from those constituents in the sample that remain unbound. Accordingly, it is respectfully submitted this basis for rejection may properly be withdrawn.

Rejection under 35 U.S.C. § 103

Claims 1, 2, 8, 13-33 and 56 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Berenson *et al.* (US 2002/0058019, hereinafter "Berenson") view of Kemshead & Ugelstad (*Mol. Cell. Biochem.* 1985, 67:11-18, hereinafter "Kemshead") and Rudi *et al.* (*Appl. Environ. Microbiol.* 1998, 64:34-37, hereinafter "Rudi").

Berenson allegedly teaches a method of separation of monocytes from blood preparations using the well-known property inherent in monocytes to engulf various materials indiscriminately. Of particular note is the teaching that the method taught by Berenson relies solely on the ability of

monocytes engulf the magnetic microbeads rather than a particular functional group. The Office acknowledges that Berenson does not teach all the limitations of the pending claims. To cure the deficiencies of Berenson, the Office cites Kemshead, which allegedly teaches the use of magnetic materials for medical applications. The Office notes that Ugelstad was one of the pioneer inventors of magnetic microbeads, and is responsible for ensuring the size homogeneity of polymeric magnetic microbeads and inclusion of derivatized magnetic microbeads further useful in medically relevant treatment and diagnostic methods, as well as usefulness in basic medical research. The Office further notes that Kemshead specifically teaches separation methods using magnetic microbeads for a variety of cell types using both non-specific binding (e.g., Section II) and specific binding partners (e.g., Section III). The Office states that the combination of Berenson and Kemshead teaches that several types of cells can be separated and enriched from model environmental and clinical samples using magnetic polystyrene beads. Additionally, Rudi is cited for its teaching of a method of using magnetic microbeads to sequentially separate bacteria from environmental samples and amplify separated DNA using the same magnetic microbeads.

The Office argues that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Berenson with the various washing and organelle preparation techniques as taught by Kemshead and/or Rudi. The skilled artisan allegedly would have been motivated to do so because of the nonspecific incorporation of beads by monocytes as taught by Berenson and the rapidity of magnetic bead separation as taught by Kemshead and/or Rudi, especially when attempting isolation from rich sources of cells such as blood, cell cultures and/or dilute environmental sources. The Office asserts that there would have been a reasonable expectation of success, given well-known phagocytic properties of monocytes as taught by Berenson and the general utility of magnetic bead separation methods as taught by many researchers especially including Kemshead and/or Rudi.

Applicants respectfully traverse this rejection for the following reasons.

The Examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532 (Fed. Cir. 1993). Only if this burden is met does the burden of coming forward with rebuttal argument or evidence shift to the applicant. *Id.* at 1532. When the references

cited by the examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988). A *prima facie* case of obviousness requires the satisfaction of three requirements. First, the combined prior art references must teach or suggest all of the claim limitations. *In re Royka*, 490 F.2d 981, 985 (CCPA 1974); MPEP § 2143.03. Second, there must be some suggestion or motivation, either in the references or in the knowledge generally available among those of ordinary skill in the art, to modify the reference. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1731 (2007). And third, there must be a reasonable expectation of success found in the prior art. *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991); MPEP § 2143.02.

The Office is respectfully reminded that claim 1 was previously amended to specify that the magnetic bead has a diameter of 200 nm and is modified to comprise a hydroxyl, a carboxyl or an epoxy group. As discussed in the previous response, the present application discloses that relatively minor changes in the size of the magnetic beads had an unexpectedly strong effect on the leukocyte separation efficiency. The optimal separation efficiency was obtained when 200 nm magnetic beads were used, with marked decreases in separation efficiency observed at less than 100 nm and more than 300 nm bead diameters. Importantly, when the surface of 200 nm magnetic beads was modified with a hydroxyl, carboxyl or epoxy groups, the leukocyte separation efficiency approached 90-100%, which had not been previously reported in the absence of a specific binding agent (e.g., an antibody).

The invention of Berenson is primarily directed to methods for stimulating cells, and more specifically, to a method for concentrating and stimulating cells that maximizes stimulation and/or proliferation of such cells (Abstract). A careful reading of Berenson reveals that it does not teach or even suggest using 200 nm magnetic beads modified to comprise a hydroxyl, a carboxyl or an epoxy group. For convenience, the relevant passages from Berenson are reproduced below:

> **[0105]** With respect to monocyte depletion noted above, monocyte populations (i.e., $CD14^+$ cells) may be depleted from blood preparations prior to ex vivo expansion by a variety of methodologies, including anti-CD14 coated beads or columns, or utilization of the phagocytotic activity of these cells to facilitate removal. Accordingly, in one embodiment, the

invention uses paramagnetic particles of a size sufficient to be engulfed by phagocytotic monocytes. In certain embodiments, the paramagnetic particles are commercially available beads, for example, those produced by Dynal AS under the trade name Dynabeads™. Exemplary Dynabeads™ in this regard are M-280, M-450, and M-500. In one aspect, other non-specific cells are removed by coating the paramagnetic particles with "irrelevant" proteins (e.g., serum proteins or antibodies). Irrelevant proteins and antibodies include those proteins and antibodies or fragments thereof that do not specifically target the T-cells to be expanded. In certain embodiments the irrelevant beads include beads coated with sheep anti-mouse antibodies, goat anti-mouse antibodies, and human serum albumin.

[0106] In brief such depletion of monocytes is performed by preincubating ficolled whole blood or apheresed peripheral blood with a one or more varieties of irrelevant or non-antibody coupled paramagnetic particles (approx. 1 vial of beads or $4x10^9$ beads to one batch of cells (typically from about $5x10^8$ to about $2x10^{10}$ cells) for about 30 minutes to 2 hours at 22 to 37 degrees C., followed by magnetic removal of cells which have attached to or engulfed the paramagnetic particles. Such separation can be performed using standard methods available in the art. For example, any magnetic separation methodology may be used including a variety of which are commercially available, (e.g., DYNAL® Magnetic Particle Concentrator (DYNAL MPC®)). Assurance of requisite depletion can be monitored by a variety of methodologies known to those of ordinary skill in the art, including flow cytometric analysis of CD14 positive cells, before and after said depletion.

[0182] In certain experiments described herein, the process referred to as XCELLERATE I™ was utilized. In brief, in this process, the XCELLERATED T-cells are manufactured from a peripheral blood mononuclear cell (PBMC) apheresis product. After collection from the patient at the clinical site, the PBMC apheresis are washed and then incubated with "uncoated" DYNABEADS® M-450 Epoxy T. During this time phagocytic cells such as monocytes ingest the beads. After the incubation, the cells and beads are processed over a MaxSep Magnetic Separator in order to remove the beads and any monocytic/phagocytic cells that are attached to the beads…

Thus, Berenson appears to disclose two basic approaches to monocyte depletion: (1) by phagocytosis of magnetic particles; and (2) by attachment of protein-coated magnetic particles. Because the second approach specifically targets particular cell types (i.e., monocytes), it falls outside the scope of claim 1, which is not concerned with specific binding. Accordingly, the Office

focuses on the phagocytosis of magnetic particles by monocytes. In doing so, the Office seems to disregard the fact that phagocytosis is a size-dependent phenomenon that is incompatible with the presently claimed method. It is well known in the art at the time of the present invention that the term "phagocytosis" generally refers to the uptake of relatively large particles (≥0.5 μm) into cells which occurs by an actin-dependent mechanism (*see*, e.g., A. Aderem and D.M. Underhill, "Mechanisms of Phagocytosis in Macrophages," *Annu. Rev. Immunol.* 1999, 17:593-623, attached herein as ***Exhibit A***; at page 594). Consistent with this notion, Berenson expressly teaches the use of Dynabeads® M-280, M-450, and M-500, all of which have diameters greater than 1 μm (*see* Dynal Biotech, "The Principles of Dynabeads®," attached herein as ***Exhibit B***; at page 10).

Moreover, a number of studies have demonstrated that 200 nm microspheres are poorly internalized by phagocytosis. In one study, polystyrene microspheres with diameters of 0.2, 0.5, 1.0, 6.0 and 10 μm were added to a HL-60 differentiated macrophage-like cell culture system, and the extent of phagocytic uptake was determined by the amount of superoxide generated from activated macrophages using a chemiluminescence (CL) assay with luminol (*see* N. Yamamoto *et al.*, "Dependence of the phagocytic uptake of polystyrene microspheres by differentiated HL60 upon the size and surface properties of the microspheres," *Colloids & Surfaces B: Biointerfaces* 2002, 25:157-162, attached herein as ***Exhibit C***). When polystyrene microspheres with diameters of 0.2 μm were added to the system, the CL intensity was low. In contrast, when polystyrene microspheres having diameters between 0.5 and 6 μm were added, the CL intensity gradually increased, with maximum internalization efficiency observed for 1 μm microspheres (*see* Yamamoto at page 161, Fig. 5 and accompanying text). Similar results were reported in a study of alveolar macrophages (*see* K. Makino *et al.*, "Phagocytic uptake of polystyrene microspheres by alveolar macrophages: effects of the size and surface properties of the microspheres," *Colloids & Surfaces B: Biointerfaces* 2003, 27:33-39, attached herein as ***Exhibit D***). Consistent with the earlier findings in HL-60 cells, it was found that when polystyrene microspheres with diameters of 0.2 μm were added to the macrophage system, the CL intensity was almost the same as that of control (PBS solution), showing that the microspheres were not internalized by the cells. The CL intensities were slightly greater for polystyrene microspheres with diameters between 0.5 and 6 μm, and that for 1 μm microspheres was the greatest (Makino at page 37, Fig. 2 and accompanying text on page 35).

Just like Berenson, Kemshead does not teach or even suggest using 200 nm magnetic beads modified to comprise a hydroxyl, a carboxyl or an epoxy group. Indeed, in Section II, which describes non-specific cell binding, no particle sizes are disclosed at all. In Section III, which is concerned with specific binding, Kemshead teaches various size ranges of magnetic beads that have been developed by different groups. The relevant passages from Section III are reproduced below (pages 13-14, emphasis added, citations omitted):

> Guesdon and Avrameas first described a preparation of magnetic particles, (Magnogel 44) which were relatively large and heterogenous in size (50-160 µm). These are hydrophilic agarose-polyacrylamide beads, containing free amino groups, enabling antibodies to be attached by simple glutaraldehyde coupling…
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> Albumin particles have been made of 0.2-2 µm diameter, that have protein A incorporated into their surface so that selective immunoglobulin isotypes will bind to their surface…
>
> Kronick et al. have used a novel polymerization protocol, involving a redox scheme to initiate methacrylate polymerisation specifically in the vicinity of magnetic [*sic*] particles. This technique results in preparation of microspheres of approximately 50 nm in diameter…
>
> At high pH, in the presence of ferromagnetic fluid, glutaraldehyde was polymerised to produce particles of 50-1500 nm diameter…
>
> Unquestionably the most monodisperse and uniform magnetic microspheres yet produced are those made by Ugelstad by the polymerisation of styrene divinylbenzene. These polystyrene particles can be prepared in uniform sizes from approx. 1.0 µm in diameter…
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> Ugelstad and co-workers have continued to modify and improve these microspheres. Recently he has grafted a further hydrophobic polymer over the surface of the polystyrene beads increasing their size from 3 to 4 µm… In addition the 4 µm coated particles of Ugelstad have epoxy groups incorporated in their surface that can be modified to enable proteins to be linked to the particles through free amino groups.

Thus, Kemshead either teaches relatively large (>1.0 μm), unmodified or epoxy-coated magnetic particles, or relatively small (nanoscale) particles coated with antibodies for specific binding. There is no specific teaching or even suggestion of 200 nm magnetic beads modified to comprise a hydroxyl, a carboxyl or an epoxy group. However, it is well settled that disclosure of a genus or range does not automatically render a particular claimed species within that genus or range obvious. When a single prior art reference discloses a genus encompassing the claimed species or subgenus but does not expressly disclose the particular claimed species or subgenus, the Office must show that the differences between the prior art primary reference and the claimed invention as a whole would have been obvious. MPEP § 2144.08. As noted above, Applicants have unexpectedly discovered that an optimal cell separation efficiency is achieved when 200 nm magnetic beads are used, with marked decreases in separation efficiency observed with less than 100 nm and more than 300 nm beads. Moreover, when the surface of 200 nm magnetic beads is modified with a hydroxyl, carboxyl or epoxy groups, the separation efficiency approaches 90-100%, which was not previously reported in the absence of a specific binding agent (e.g., an antibody). Accordingly, the claimed combination of magnetic bead size and surface modification is non-obvious in view of prior art.

Thus, the combination of Berenson and Kemshead fails to disclose each and every element of the claimed invention. Even if we assume, *arguendo*, that the combination of Berenson and Kemshead teaches all the elements of claim 1 as amended, there is no motivation to combine the teachings of Berenson and Kemshead to arrive at the presently claimed method with a reasonable expectation of success. As noted above, Berenson teaches the use of magnetic microparticles (>1.0 μm) for monocyte depletion. The prior art teaches that 200 nm particles are not internalized to an extent sufficient to facilitate magnetic sorting. In light of this unequivocal teaching away, it is unclear what could possibly have motivated a person skilled in the art at the time of the invention to modify the nonspecific phagocytic uptake of magnetic microparticles taught in Berenson to arrive at the presently claimed method with a reasonable expectation of success.

Rudi was cited solely for the proposition that the same magnetic beads may be used to sequentially separate bacteria from a sample and amplify bacterial DNA. As such, Rudi does not cure the deficiencies of Berenson and Kemshead identified above.

Because the cited combinations of prior art references fail to teach all the elements of the claimed invention, and a person skilled in the art could not have been motivated to combine the teachings of Berenson and Kemshead and/or Rudi with a reasonable expectation of success in view of the knowledge available in the art at the time of the invention, it is respectfully submitted that the Office has failed to establish a *prima facie* case of obviousness. Accordingly, Applicants request that this rejection under 35 U.S.C. § 103(a) be withdrawn and the application be passed to issue.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing Docket No. 514572000600.

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